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Efficacy of Endometrial Cytology to Diagnose Subclinical Endometritis in Repeat Breeder Cows

Vinay Kumar Manjhi*, Nitin Kumar Bajaj, Suresh Prasad Shukla, Pratipal Singh Kaurav and Ravi Kumar Thakur

Department of Veterinary Gynaecology & Obstetrics, College of Veterinary Science & Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Rewa, M.P., INDIA

**Corresponding author: VK Manjhi; Email: vinaymanjhi54@gmail.com*

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ABSTRACT

A total of 500 cows included in the study were randomly selected from college livestock farm, Kuthuliya and different villages in and around Rewa (M.P.). On the basis of characteristics of cervico-vaginal mucus (CVM), per-rectal examination, Whiteside test and endometrial cytology by cytobrush technique, the prevalence of clinical and subclinical endometritis in these repeat breeder cows was recorded as 16.00 (80/500) and 28.00 (140/500) per cent, respectively. Endometrial cytology revealed polymorphonuclear cell (PMN) per cent in normal, clinical and subclinical endometritis groups to be 4.00±0.03, 34.80±0.25 and 14.02±0.14 per cent, respectively and difference between them was significant ($p<0.05$). It was concluded that the prevalence of subclinical endometritis in repeat breeder cows was recorded as 28.00 (140/500) per cent. Endometrial cytology by cytobrush technique is effective diagnostic technique for diagnosis of subclinical endometritis in repeat breeder cows at field level.

Keywords: Subclinical endometritis, Whiteside test, endometrial cytology, Repeat breeder cows

Several studies have reported variations in the prevalence of uterine infections (Plontzke *et al*., 2010). The variations in prevalence rates have been attributed to different ways of classification, management systems, breed differences, environmental factors, nutrition, age and parity amongst others (Gautam *et al*., 2010). Prevalence of uterine infections varies from 3.3 per cent to 23.6% (Gautam *et al*., 2010). Uterine infections include pyometra, mucometra, endometritis, metritis and tumors. Among all of these, repeat breeding due to endometritis is one of the major gynaecological problem affecting reproductive efficiency and economy of milk production in animals. Good fertility in dairy cows is considered as key to economically successful dairy farming. It is widely accepted that uterine disorders in the postpartum period have a negative impact on reproductive performance. The prevalence of subclinical endometritis in repeat breeder cows is 12.70 per cent (Pothmann *et al*., 2015).

Repeat breeding syndrome results in lowered dairy profit via wastage of semen and increased insemination cost, increasing intervals to conception, increasing culling and replacement costs, losing genetic gain through increased generation intervals and reducing fertility. After the postpartum period, repeat breeding is considered one of the most important reproductive disorders in cattle (Yusuf *et al*., 2010) which affect reproductive efficiency. Clinical and subclinical endometritis are common causes of infertility and subfertility in high producing dairy cows, delaying the onset of ovarian cyclic activity after parturition, extending luteal phases and reducing conception rates (Sheldon *et al*., 2009).

Routine methods used for diagnosing endometritis involve uterine biopsies, lavage, but these may cause irritation and distortion of cells. An inconsistent success following conventional therapies is achieved due to lack of diagnostic standards. Therefore, the recent studies have been focused on sophisticated diagnosis of endometrial alterations beyond clinical signs of endometritis. A novel approach

for uterine cytological examination is cytobrush technique which is considered as a reliable method in dairy animals (Bajaj *et al.,* 2016a).

Keeping this in view, the present study was planned with an objective to study the prevalence of repeat breeding due to subclinical endometritis in cows and to investigate the efficacy of cytobrush technique in diagnosing subclinical endometritis in cows.

MATERIALS AND METHODS

A total 500 cows were randomly selected from college livestock farm, Kuthuliya and different villages in and around Rewa (M.P.). After recording history all the animals were subjected to gynaeco-clinical examination, Whiteside test and endometrial cytology by cytobrush technique.

Sample collection

Samples for endometrial cytology were collected from all the animals and those found positive for subclinical endometritis (>5 per cent PMNs for >47 days postpartum) were subjected to bacterial isolation and culture sensitivity. After proper restraining, the animals were subjected to evacuation of rectum through back racking. The perineal region and vulva were washed with savlon and water and later on disinfected with spirit swab. The vulvar lips were pulled apart by an assistant and the modified cytobrush assembly (Madoz *et al*., 2014). A stainless steel device was attached with a sterile endocervical brush used for Pap smear test in humans. The device was covered with sterile plastic sheath to prevent contamination from vaginal discharges. At the external Os of the cervix the outer plastic sheath was perforated and stainless steel guard and cytobrush was passed into and through the cervix. Upon reaching the uterine body, the cytobrush was advanced beyond the stainless steel guard into the lumen of the uterine body where it was rotated clockwise (360 degrees) to obtain cellular material from the endometrium. The cytobrush and rod was retracted inside the guard and carefully removed from the reproductive tract. The threshold cut off values for diagnosis of subclinical endometritis by endometrial cytology were more than 5 per cent PMNs as described by Gilbert *et al.* (2005).

Staining method for endometrial cytology

Immediately after removal from reproductive tract, the sample taken for cytological analysis was rolled over a glass microscopic slide, air dried and transported to the lab for examination. The cells were fixed and stained with modified Wright-Giemsa stain and examined under microscope at 400x magnification to identify individual cell types including endometrial cells and polymorphonuclear cells (PMN). A total of 300 cells were counted and PMN cell count was expressed as the proportion of PMN cells counted out of combined PMNs plus endometrial cells. This data was serve to classify the health status of uterus along with nature of discharge (clear, purulent or mucopurulent) as clinical or subclinical endometritis or normal (without inflammation or healthy). The threshold cut of values for endometrial cytology were similar as described by Gilbert *et al.,* 2005 (>5 per cent PMNs for >47 days postpartum).

All the animals were subjected to aseptic collection of uterine fluid and endometrial tissue by low volume lavage technique. The aseptically collected fluid from all the animals were subjected to culture isolation and identification of bacterial micro-organisms as per the method described by Cruickshank, 1965.

Statistical analysis

The data was analysed using SYSTAT software, Version 12, San-Jose California USA. Data from experiment was presented as Mean ± SE. The pair-wise comparison of means was carried out using Fisher's multiple comparison tests as per standard statistical method described by Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

On the basis of characteristics of cervico-vaginal mucus (CVM), per-rectal examination, Whiteside test and endometrial cytology by cytobrush technique, 44.00 (220/500) per cent repeat breeder cows were found to be suffering from endometritis. The prevalence of clinical and subclinical endometritis in these repeat breeder cows was recorded as 16.00 (80/500) and 28.00 (140/500) per cent, respectively.

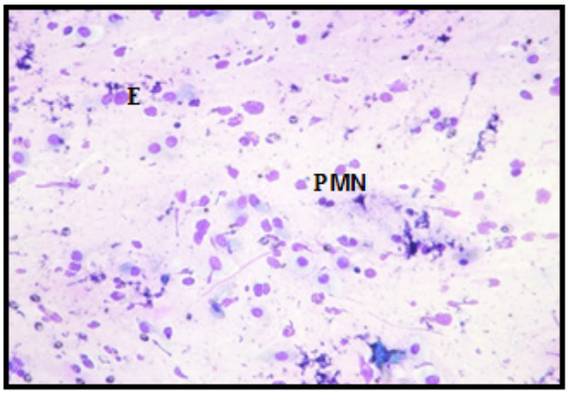

Fig. 1: Endometrial smear by cytobrush technique from subclinical endometritic cows showing Polymorphonuclear cells (PMN) and endometrial cells (E). Modified Wright Giemsa stain X 400

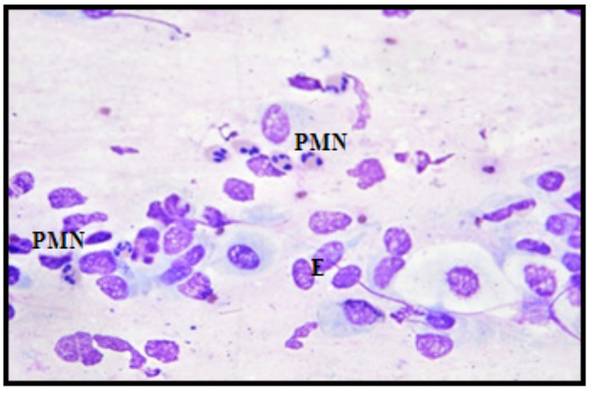

Fig. 2: Endometrial smear by cytobrush technique from subclinical endometritic cows showing Polymorphonuclear cells (PMN) and endometrial cells (E). Modified Wright Giemsa stain X 1000

The above findings in the present study are almost similar to the findings of Singh *et al.* (2016) who reported the prevalence of subclinical endometritis in repeat breeder cows to be 29.40 per cent. The lower prevalence (12.70%) of subclinical endometritis in repeat breeder cows was reported by Pothmann *et al.* (2015). However, higher prevalence was also reported by Salasel *et al.* (2010) as 52.7 per cent. Drillich *et al*. (2005) reported the prevalence of clinical endometritis (CE) and subclinical endometritis (SCE) 18 to 37 per cent. Bajaj (2015) also reported the prevalence of subclinical endometritis in postpartum Murrah buffaloes to be 26.00 per cent.

All the 500 cows were subjected to endometrial cytology by cytobrush technique and results were recorded in the form of per cent polymorphonuclear cell. The detailed results of endometrial cytology by cytobrush technique are tabulated in table 01. Endometrial cytology by cytobrush technique in different groups (normal, clinical and subclinical endometritic cows) revealed polymorphonuclear cell (PMN) percentage to be 4.00±0.03, 34.80±0.25 and 14.02±0.14, respectively. The difference between the groups for PMN cell percentage was found to be significant ($p<0.05$). The mean of fibroblasts value was recorded as 0.30±0.05 and 14.00±0.14 in subclinical and clinical endometritic cows, respectively. Fibroblasts were not observed in endometrial smears of normal cows.

Polymorphonuclear cells (PMN) are the predominant inflammatory cell types found in intrauterine fluid accumulations and determination of the relative proportion of PMN has been observed to be predictors of reproductive performance in the postpartum cows.

In present study, endometrial cytology by cytobrush technique revealed significant difference ($p<0.05$) in PMN cell percentage in normal, clinical and subclinical endometritic cows. However, the PMN percentage was significantly higher clinical endometritic cows as compared to subclinical endometritic cows. Honparkhe *et al.* (2014) found that 5 per cent neutrophils/PMN at >47 days postpartum as an indicator of subclinical endometritis in cattle.

However, PMN cell percentage in normal, clinical and subclinical endometritic buffaloes was reported by Bajaj *et al.* (2016b) as 4.34±1.85, 35.35±3.43 and 21.17±0.45 per cent, respectively.

The cows found positive for subclinical endometritis on the basis of various diagnostic tests performed alone were also subjected to bacterial isolation. The distribution of cows on the basis of endometrial cytology by cytobrush technique alone was taken as the base and considered to be 100 per cent positive for determining efficacy of this diagnostic technique. When the uterine lavage samples obtained from cows were examined for microbial assay in addition to endometrial cytology by cytobrush technique, out of 140 repeat breeder cows, 112 (80.00%) were found to be positive.

A combination of EC along with microbial assay will help in ruling out non-infectious cytologic endometritic cases. These findings prove that efficacy of endometrial cytology to detect false positive cases was increased when microbial assay was combined with endometrial cytology.

According to Barlund *et al.* (2008) two populations of postpartum cows exist which apparently appear normal. One with impaired uterine clearance (good volume of fluid in lumen) and other with increased inflammatory response (lower or no fluid volume in lumen). Animal with increased inflammatory response may be without infection or infection might be spontaneously cleared off by their natural defence mechanism.

CONCLUSION

Endometrial cytology by cytobrush technique is effective and standard diagnostic technique for diagnosis of subclinical endometritis at field level. Efficacy and precision of endometrial cytology by cytobrush technique increases when used in combination with microbial assay for diagnosis of subclinical endometritic repeat breeder cows.

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